Award Number:
W81XWH-09-2-0109

TITLE:
Innovative Computational Waterborne Pathogen Research for Chemical/Biological Detection

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REPORT DATE:
September 2010

TYPE OF REPORT:
Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
01-09-2010	Annual	15 August 2009 - 14 August 2010

4. TITLE AND SUBTITLE

Innovative Computational Waterborne Pathogen Research for Chemical/Biological Detection

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-09-2-0109

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Maryland
College Park, MD 20742

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

We are developing a device consisting of multiple parallel microcantilevers that will be used to detect pathogens in fluid-based environments. These microfabricated devices will allow water samples to be surveyed and monitored in real time. They will be highly sensitive and extremely accurate. The parallel MEMS cantilever-based platform that we are developing is based on nucleic acid pathogen signatures that are identified using software that we have developed. The initial prototype devices have low background and exquisite sensitivity to test target sequences. The completed devices are expected to allow the detection of small numbers of pathogens with a high degree of confidence. With the combined expertise of life scientists, engineers, and computational biologists, MPRI is uniquely positioned to develop, expand, and oversee this project.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	10	19b. TELEPHONE NUMBER *(include area code)*

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Annual Report:

Introduction:

We are constructing a prototype device to detect waterborne pathogens in real time, with a high degree of sensitivity, and low false positives. In the next year, we will continue refining this device with the goal of β-testing it against a limited number of known bacterial species. This will allow us to determine the feasibility of this approach, the sensitivity of this instrumentation, and the specificity of detection. The milestones that have been completed thus far include:

–DNA probe chemically immobilized on microchip surface.

–Target sequences in sample hybridize at surface with probes.

–Enzyme-labeled sandwich probe hybridizes to target sequence.

–Sensitivity has been improved so that as little as 0.5 nM of target DNA sequences can be recognized in microchannels with interdigitated microelectrodes

Body:

The goal of this project is to construct an on-chip DNA biosensor that will be used to detect pathogens in contaminated water. The on-chip format enables integration with other functions, such as sample processing, DNA extraction and PCR. The unique design will have several biosensors fabricated in parallel so that it will be possible to detect multiple signatures from a single species, as well as multiple species/strains with a single device (Figure 1). The novel aspects of the pathogen detection device that we propose to develop are that i) they have a high degree of pathogen specificity, ii) they are highly sensitive, iii) they can detect pathogens in real time, iv) and they have virtually no false positives.

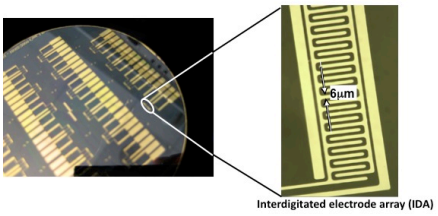


Figure 1. Schematic of biosensor device designed to detect multiple pathogens at the same time.

Signature selection. For the initial proof of concept studies, the bacterium *P. aeruginosa* is being used for detection, because it is a well-understood model organism with both pathogenic and non-pathogenic subtypes. Using the Insignia pipeline (Figure 2) we have selected signature sequences for our initial testing. We have generated signatures that distinguish pathogenic from nonpathogenic strains. We have also designed and had synthesized targets with one or two mismatches and the probes that are complementary to those targets (Figure 3). Experiments using these oligos will further test the specificity of the probes binding to the targets.

We have also made significant updates in bioinformatics, specifically to the Insignia Pipeline database. In the past year, Dr. Steven Salzberg, one of the main contributors on this project and the developer of Insignia, has increased the number of pathogens and their related signatures in the database from 8,341 to 13,928. The implications of this significant increase are that we will be able to adapt the biosensor to recognize an extensive range of pathogens.

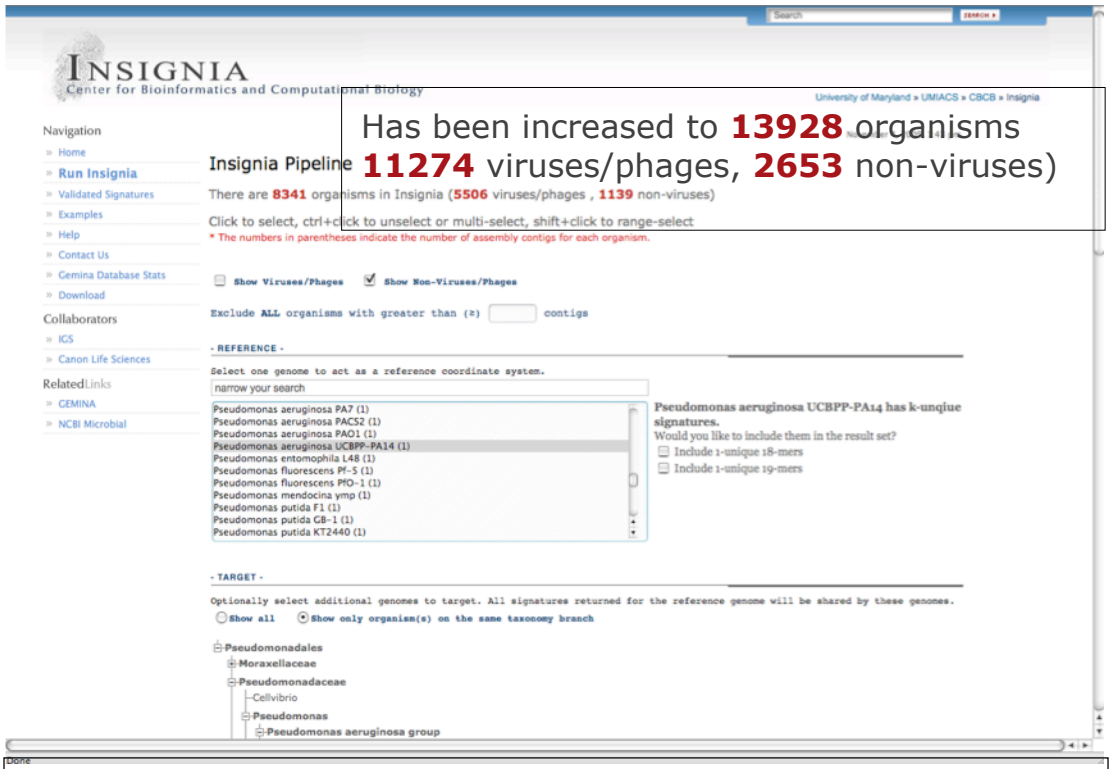


Figure 2. Insignia Pipeline.

Figure 3. Signatures designed for specificity testing in prototype waterborne pathogen detection device.

Wildtype:
5′GTTGCCCTGGACATTGATCTGGATGTTGTTGCTTTCCATCG3′

Target 1:
5′GTTGCCCTGGACATTGATCTGGATGTTGTTGGTTTCCATCG3′

Target 2:
5′GTTGCCCTGCACATTGATCTGGATGTTGTTGCTTTCCATCG3′

Target 3:
5′GTTGCCCTGCACATTGATCTGGATGTTGTTGGTTTCCATCG3′

Probe A:
5′-NH2CGATGGAAAGCAACAACATC3′

Probe A variant:
5′-NH2CGATGGAAACCAACAACATC3′

Probe B:
5′AGATCAATGTCCAGGGCAAC3′-Thiol

Probe B variant:
5′AGATCAATGTGCAGGGCAAC3′-Thiol

In the first year, we have developed and then continued refining the pathogen biosensor. Figure 4 depicts the binding of the oligonucleotide probe to the biosensor and the signal detection methodology. This includes that the DNA probe chemically immobilized on microchip surface and then target sequences in each sample hybridize at surface with probes. An enzyme-labeled sandwich probe hybridizes to target sequence. Key to the detection is that the substrate (PAPG) is cleaved by enzyme (β-gal) and forms PAP. Since PAP and PQI are reversible states of redox active molecules, each redox event (PAP -> PQI, PQI -> PAP) results in a current signal thereby amplifying the signal (Figure 5).

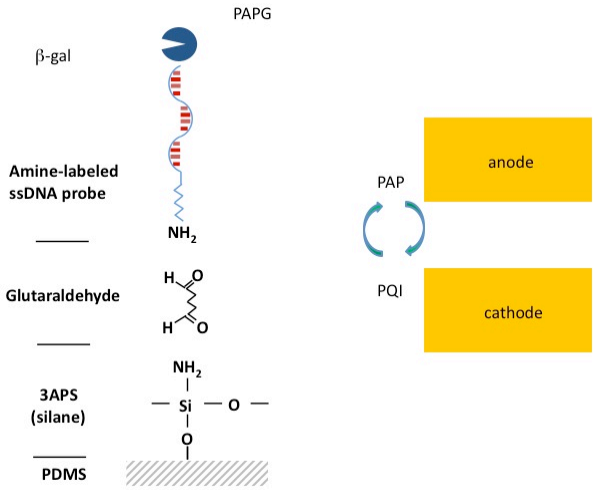
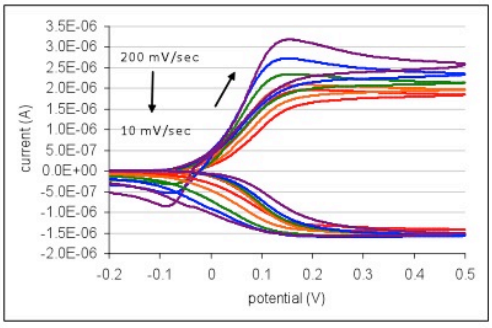


Figure 4. Schematic of on-chip pathogen biosensor.



Cyclic voltammograms of 1 mM 4-aminophenol (PAP) solution on Au IDAs at 10, 20, 50, 100 and 200 mV/sec in 35-µL sample droplets. Collecting electrode (cathode) was kept at -0.15 V.

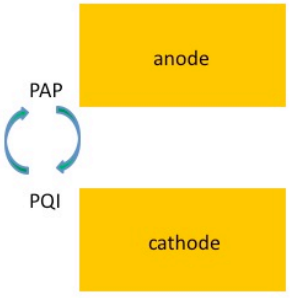
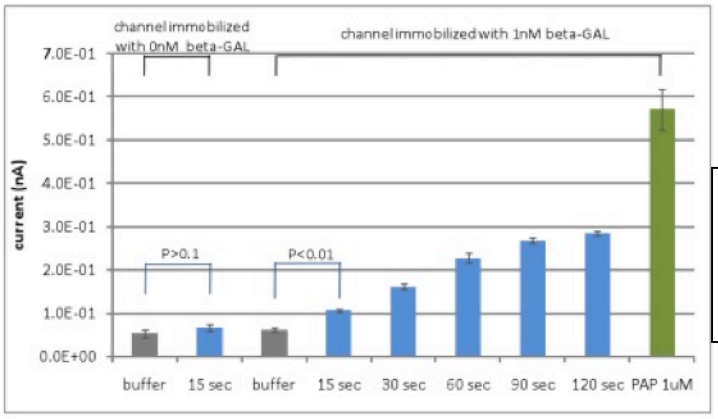


Figure 5. Characterization of interdigitated microelectrodes.

Initial experiments in Q1 that 100 nM of target DNA can bind to specific labeled probe and elicit biosensor current above background. We have recently demonstrated that as little as 0.5 nM of target DNA sequences can be recognized in microchannels with interdigitated microelectrodes (Figure 6). This represents a 200-fold improvement since this project began in August of 2009. We are continuing to refine the detection system to increase the sensitivity.



Oxidation current recorded on IDA in microchannel immobilized with 0 and 1nM β-GAL.

Figure 5. Detection of surface confined β-gal in microchannel with interdigitated microelectrodes.

Next steps will include empirically determining optimal signature length. Several signatures that are unique to a selected pathogen and of the appropriate length will be used to develop a panel of pathogen-specific oligonucleotide probes. We aim to perform the entire biosensing experiment on electrode microchip and determine detection limits for the specific DNA sequence. We also plan to test strategies for optimizing selective hybridization.

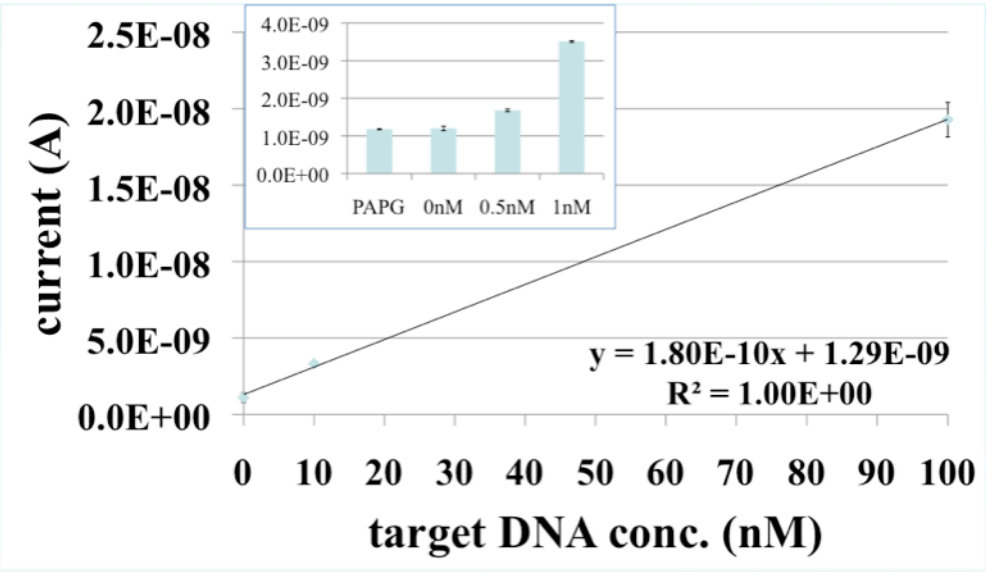


Figure 6. Nanomolar detection of target DNA sequences. The sequence recognition follows a linear signal response vs. target sequence concentration.

Problems:

1) Sample preparation: Samples may need to be concentrated in order to maximize exposure of the devices to large quantities of water.

Dr. Ian White in the Bioengineering Department is currently working on methods to filter and concentrate samples efficiently so that pathogens that may be contaminating the samples can effectively be detected.

2) Vibration. Handheld devices and even stationary devices are subject to mechanical vibration and physical movement that can significantly impact the system's performance and reliability.

Drs. Bentley and Payne and others in the Bioengineering Department are currently working on this issue, and will make every effort to design the proposed detection devices with maximum reliability and stability.

Key Research Accomplishments:

- Developed DNA signatures for *P. aeruginosa*
- Developed first generation of microchannel biosensor with interdigitated microelectrode
- Made significant upgrades to Insignia Pipeline

Reportable outcomes:
Upgraded Insignia Pipeline (open source searchable database, http://insignia.cbcb.umd.edu/)

Conclusion:
We have made progess in developing a biosensor that can detect pathogens in contaminated water. This biosensor will be extremely sensitive, inexpensive to produce and have negligible false positive or negative results. We are in the process of further improving the detection limits and determining the specificity. In the next year, we will continue refining the device for β-testing.

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Research and Development Timeline:

	Start Date	Completed	Remaining
Develop detection device and sensors	11/1/09	305	240
Identify pathogen signatures and generate oligonucleotides	8/15/09	180	0
Optimize linking oligos to cantilevers	3/15/10	180	185
Validate pathogen signatures	1/1/11	0	365
Culture bacterial strains and prepare DNA for detection testing	3/1/11	0	180
Test detecition devices and modify as needed	8/15/11	0	365
Quantitate sensitivity and specificity of devices	8/15/11	0	365
Develop and optimize sample preparation tenchniques	1/1/12	0	365